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BIOCONCENTRATION AND METABOLISM OF [^{14}C]3-CHLORO-*P*-TOLUIDINE HYDROCHLORIDE BY BLUEGILL SUNFISH

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Abstract—Bluegill sunfish (*Lepomis macrochirus*) were continuously exposed to 0.1 μg/ml of uniformly radiolabeled [^{14}C]3-chloro-*p*-toluidine hydrochloride (CPTH) for 28 d, after which the fish were transferred to flowing untreated water for a 28-d depuration period. At periodic intervals during the treatment and depuration periods, samples of water and fish were analyzed for [^{14}C] residues to determine the degree of CPTH absorption. Samples of bluegill that were exposed to the radiolabeled chemical for 28 d were analyzed to determine the distribution of the radiolabel and to isolate and identify possible metabolites. The steady-state concentrations in the bluegill tissues were reached by day 7 of exposure to the radiolabeled compound, with mean concentrations in the edible, nonedible, and whole-body tissues determined to be 2.9, 12.0, and 7.5 μg/g of tissue, respectively. Bioconcentration factors of 33×, 150×, and 88× were calculated for the three types of tissues. About 64% of the accumulated radiolabeled CPTH and metabolites were eliminated from the fish on day 28 of depuration. The distribution of radioactivity was found to be similar in all tissue groups. One metabolite was confirmed as *N*-acetyl-3-chloro-*p*-toluidine, with two metabolites suggested to be 4-acetamido-2-chlorobenzoic acid and 4-amino-2-chlorobenzoic acid. The *N*-acetylated breakdown product has been previously reported to occur in both mammals and birds; thus, the metabolism of CPTH in fish may parallel that observed in these other species. Although the effects of biomagnification cannot be fully assessed at this time, we can conclude that when bluegill sunfish are continuously exposed to sublethal levels of CPTH, bioconcentration will not occur to an appreciable extent, and that residues will be rapidly excreted upon removal of the fish from the CPTH source. Furthermore, published information pertaining to various aquatic organisms indicates that bioaccumulation will not be a problem if the bioconcentration factor is below 100.

Keywords—Bioconcentration Metabolism Pesticide CPTH Fish

INTRODUCTION

The compound 3-chloro-*p*-toluidine hydrochloride (CPTH) has been demonstrated to be an effective and unique minor-use pesticide because of its selective high toxicity to most pest bird species, low-to-moderate toxicity to the majority of predatory birds and mammals, and a lack of known secondary hazards when used in baits. The mode of action of CPTH in starlings has been suggested to be that of a nephrotoxin [1]. The acute toxicity (oral LD50) of the avicide to starlings is reported to be between 4 and 5 mg/kg of body weight [2]. Although the primary metabolites of CPTH are indicated to be of low toxicity to birds and mammals [3], with toxicity information available for aquatic invertebrates and frog larvae [4] and shrimp/crabs [5], data are unavailable in the literature regarding the metabolism of the compound in fish.

Under field conditions, where the avicide is applied to soils as a seed or pellet bait to control various pest bird species (e.g., starlings or blackbirds), some of the compound is undoubtedly solubilized. However, limited data are available regarding the fate of the compound in soil and aqueous systems and the potential for absorption by fish. In response to various questions and concerns and to comply with U.S. Environmental Protection Agency (EPA) reregistration data requirements, a series of environmental fate studies were conducted at our laboratory.

The study presented in this paper describes the bioconcentration and metabolism of [^{14}C]CPTH in bluegill sunfish after continuous exposure to 0.1 μg/ml of the radiolabeled compound in an aqueous medium.

MATERIALS AND METHODS

CPTH bioconcentration phase

Test chemical. Nonradiolabeled CPTH (Purina Mills, Bridgeton, MO, USA), purity of 99%, and uniformly ring-labeled [^{14}C]CPTH (Sigma Chemical Company, St. Louis, MO, USA), specific activity of 2.25 mCi/mmol, were combined and dissolved in water and used as the stock treatment solution; purity of the radiolabeled compound was > 98% as determined by HPLC. The concentration and specific activity of the stock solution, as determined by HPLC/UV and radiometric detection (RAM), were 9.43 mg/ml and 809.6 dpm/μg (65.5 μCi/mmol), respectively.

As reported by S. Laut (1990, unpublished data), the chemical has an estimated pK_a of 3.9 for protonation of the amino group. Thus, in various media (including fish) at pH values >4.9, more than 90% of the compound will be in the free amine form (3-chloro-*p*-toluidine [CPT]). Conversely, CPTH will be present in significant amounts only in acidic media below pH 3.

Test organism and treatment level. Bluegill sunfish were obtained from the Osage Catfish Hatchery (Osage Beach, MO, USA). Prior to conducting the study, the fish were held about 14 d in a 1,700-L metal tank containing aerated well water, under a photoperiod of 16 h light and 8 h darkness. During this holding period, a representative sample of the fish population (30) was measured and weighed, with the following results: mean weight, 1.2 g (0.74–2.00 g); and mean length, 48 mm (40–56 mm). The

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Table 1. The [^{14}C] residue concentrations in exposure water and fish tissues during treatment and depuration periods

Sampling period (days)	[^{14}C] Residue concentrations[a]			
	Water (mean μg/ml ± SD)	Tissue (mean μg/g ± SD)		
		Edible (muscle)	Nonedible (carcass/viscera)	Whole body
Exposure period				
0	0.095 ± 0.002	N/S[b]	N/S	N/S
1	0.097 ± 0.004	1.6 ± 0.5	5.1 ± 1.0	3.2 ± 0.3
3	0.098 ± 0.004	1.9 ± 0.5	11 ± <1.0	6.0 ± 0.4
7	0.099 ± 0.001	2.9 ± 0.6	12 ± 2.0	7.5 ± 1.1
10	0.100 ± <0.001	3.1 ± 0.6	15 ± 1.0	8.8 ± 1.7
14	0.096 ± <0.001	3.6 ± 0.9	16 ± 2.0	9.1 ± 1.3
21	0.097 ± 0.002	3.0 ± 1.0	15 ± 3.0	8.3 ± 1.9
28	0.095 ± 0.002	3.5 ± 1.2	17 ± 3.0	9.3 ± 1.8
Depuration period				
1	<MDL[c]	2.5 ± 1.5	13 ± 9.6	7.0 ± 4.4
3	<MDL	2.1 ± 1.0	8.8 ± 4.7	4.9 ± 2.3
7	<MDL	1.7 ± 1.3	12 ± 4.2	6.2 ± 2.4
10	<MDL	2.2 ± 1.3	8.9 ± 5.2	5.0 ± 2.9
14	<MDL	1.6 ± 1.1	8.5 ± 3.2	4.7 ± 2.2
21	<MDL	1.7 ± 1.1	11 ± 6.0	5.8 ± 3.0
28	<MDL	1.6 ± 0.3	6.1 ± 1.1	3.4 ± 0.6

[a] Radioactivity in triplicate samples calculated as 3-chloro-*p*-toluidine hydrochloride equivalents.
[b] Not sampled.
[c] Minimum detection level.

fish were fed a commercial dry, pelleted food daily, ad libitum, except during 24 h prior to testing. Mortality of the population was <1% during a 48-h period before study initiation.

The acute toxicity (LC50) of CPTH to sunfish was determined prior to conducting the study, because data were unavailable in the literature. An LC50 value of 10 μg/ml was estimated under static renewal test conditions using nonlinear interpolation, with a no-observed-effect concentration established at about 4 μg/ml (M.K. Collins, unpublished data). Using these data, a concentration of 0.1 μg CPTH per milliliter of solution was chosen, which was anticipated to be sufficiently dilute so as to minimize possible sublethal toxic effects to the fish, yet afford a sufficiently high concentration of CPTH for identification of radiolabeled residues in the tissues.

Test chambers and delivery system. The glass aquaria test chambers (treated and control) were constructed using silicone adhesive and had the following dimensions: 75 × 39 × 30 cm (length × width × height). Water level in each aquarium was maintained at a depth of 25 cm for a total solution volume of 73 L. Illumination was provided by Vita-Lite® (Durotest, Boston, MA, USA), fluorescent tubes located over each aquarium; 16 h of light were provided each day. The aquaria were maintained in a temperature-controlled water bath at a temperature of 17 ± 1°C. Specific parameters of the dilution water were: total hardness, 23 to 28 mg/L; alkalinity (as $CaCO_3$), 20 to 23 mg/L; pH, 6.9 to 7.1; and specific conductance, 110 to 130 μmhos/cm.

The delivery system consisted of a Sage® (Sage Instruments, Plainview, NY, USA), syringe pump equipped with two 20-ml gas-tight syringes, each of which was calibrated to deliver 4.2 μl/min of the CPTH stock solution to a flow of 420 ml/min of dilution water, which resulted in an overall concentration of about 0.1 μg/ml of CPTH (Table 1) during the exposure period. A continuous flow diluter, similar to that described by Benoit et al. [6], was calibrated to deliver 420 ml/min of treated solution or control water to each aquarium. This delivery rate was equivalent to 8.3 aquarium volumes/d or a 90% aquarium replacement/6 h [7].

Study initiation and monitoring. Initially, 200 bluegill were placed into each of two aquaria, or exposure chambers, for determination of the bioconcentration factor and metabolite identification; a third chamber was maintained as a control. A total fish biomass of 240 g was contained in each aquarium, which decreased throughout the study as fish were removed for tissue residue analysis. During the study fish were fed a dry, pelleted food twice daily at the rate of about 2% of their total biomass per feeding, except during each 24-h period prior to sampling. Daily observations were made as to the appearance and behavior of the fish; also, the temperature and dissolved

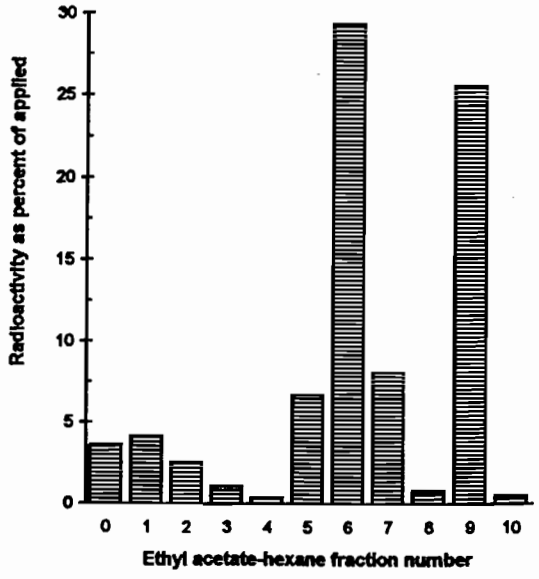

Fig. 1. Distribution of radioactivity in edible tissue extracts.

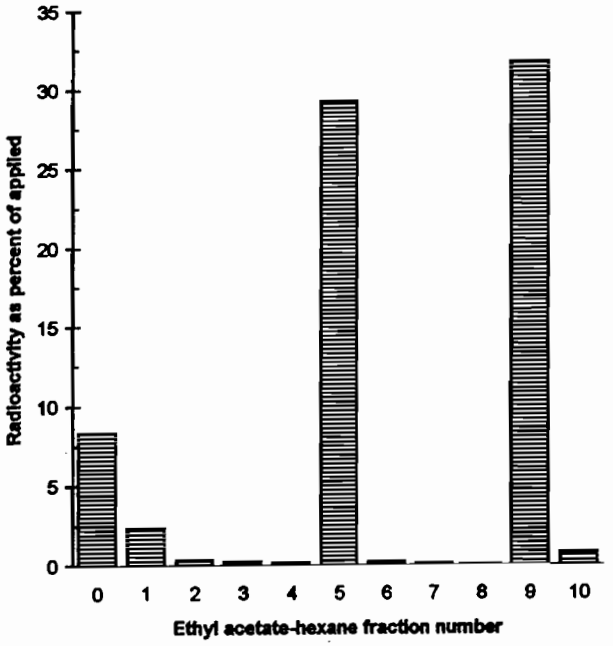

Fig. 2. Distribution of radioactivity in viscera tissue extracts.

oxygen concentrations were determined daily in each aquarium. Water hardness and alkalinity were determined at the beginning of the study [8].

The bluegill were exposed to a continuous concentration of about 0.1 μg/ml for 28 d. The establishment of an equilibrium or steady state tissue concentration (i.e., [^{14}C] tissue residues not significantly different at three consecutive sampling intervals), was determined by analysis of variance ($p = 0.05$), and Tukey's test [9,10]. Once equilibrium had been established and the fish exposed for 28 d, 35 fish were transferred from one of the treated chambers to a depuration (withdrawal) chamber, into which untreated dilution water was introduced at a rate equal to that during the exposure period. A 28-d depuration period was then maintained to establish the rate of tissue [^{14}C] residue elimination.

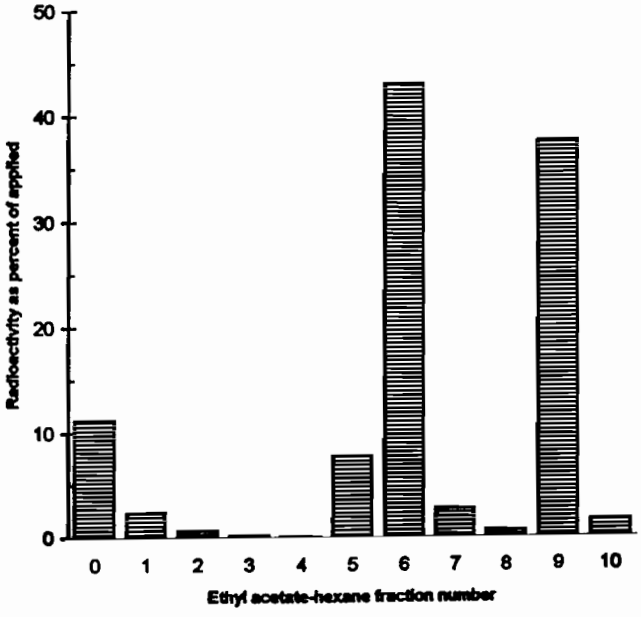

Fig. 3. Distribution of radioactivity in carcass tissue extracts.

[^{14}C] residue determinations. Water samples (5 ml) were collected from the bioconcentration and metabolite aquaria for liquid scintillation counting (LSC) on days 0, 1, 3, 7, 10, 14, and 28 of exposure, and on days 1, 3, 7, 10, 14, 21, and 28 of depuration; samples were also collected from the control aquarium on days 0 and 28 of exposure, and days 14, 21, and 28 of depuration. Each 5-ml aliquot was transferred to a scintillation vial, followed by the addition of 15 ml of Monophase S® prior to analysis by LSC. In addition, water samples were analyzed for CPTH using HPLC/UV on days 7, 21, and 28 of exposure, with the radiochemical purity and verification of [^{14}C]CPTH determined radiometrically using HPLC/RAM on day 28 of the exposure period.

Using HPLC/UV, water samples were analyzed by direct injection; however, an initial ethyl acetate partitioning of the samples was required prior to HPLC/RAM radiometric analysis. To prevent losses of the free amine form of CPTH (i.e., CPT [pK_a = 3.9]), a small volume of 1 N HCl was added to the ethyl acetate extracts prior to concentration. The concentrated samples were neutralized with 10 N NaOH prior to analysis.

During the exposure period, five fish were collected and pooled at days 1, 3, 7, 10, 14, 21, and 28, and on days 1, 3, 7, 10, 14, 21, and 28 of depuration; control fish, collected at days 0 and 28 of exposure and days 14, 21, and 28 of depuration, were analyzed for background [^{14}C] activity.

All fish in the [^{14}C]CPTH aquarium to be used for metabolite isolation and identification were removed on day 28 of the exposure period, dissected into edible (muscle), viscera, and carcass tissues. These samples were held frozen until analyzed.

Fish collected for the bioconcentration determination were dissected into edible (muscle) and inedible (viscera/carcass) tissues. These samples (representing the entire individual tissue portion for each of the fish) were air-dried for a minimum of 24 h at ambient temperature, placed into a Combusto-Cone® (Packard Instrument Company), and weighed. To aid combustion, approximately 0.2 g of cellulose powder (Whatman CC41) was added to each sample cone. Triplicate samples of each representative tissue were combusted in a Packard Model 306 or 307 Sample Oxidizer, with the resulting $^{14}CO_2$ trapped as a carbonate salt in Carbosorb® and flushed into a glass scintillation vial with Permafluor E®, followed by analysis by LSC. Oxidizer recovery rates using a standard reference material (Spec Chec [^{14}C] standard), ranged from 97 to 102%.

Liquid scintillation counting efficiencies of all study samples were determined using a company-prepared external standard (Beckman Instruments) calibration curve, with the samples counted for a maximum 100 min or until a 2 sigma error of 5% was obtained. The minimum detectable [^{14}C] residue concentration varied as to type of sample; limits of 0.015 μg/ml and 0.145 μg/g were obtained for water and tissue samples, respectively, based upon a specific activity for the radiolabeled CPTH of 809.6 dpm/μg.

The HPLC operating conditions with RAM detector were: column, Beckman Ultrasphere ODS® (5 μm), 250 × 4.6-mm i.d.; mobile phase, 58% acetonitrile:42% NANOpure® water; mobile phase flow rate, 1.0 ml/min; injection volume, 200 μl; flow cell type, liquid; flow cell volume, 800 μl; cocktail, Beckman Ready Flow III®; and cocktail flow rate, 3.0 ml/min.

Using UV dete tion, the HPLC operating conditions were: column, Beckman Ultrasphere ODS (5 μm), 250 × 4.6-mm i.d.; mobile phase, 58% acetonitrile:42% NANOpure water; flow rate, 1.0 ml/min; wavelength, 241 nm; injection volume, 150

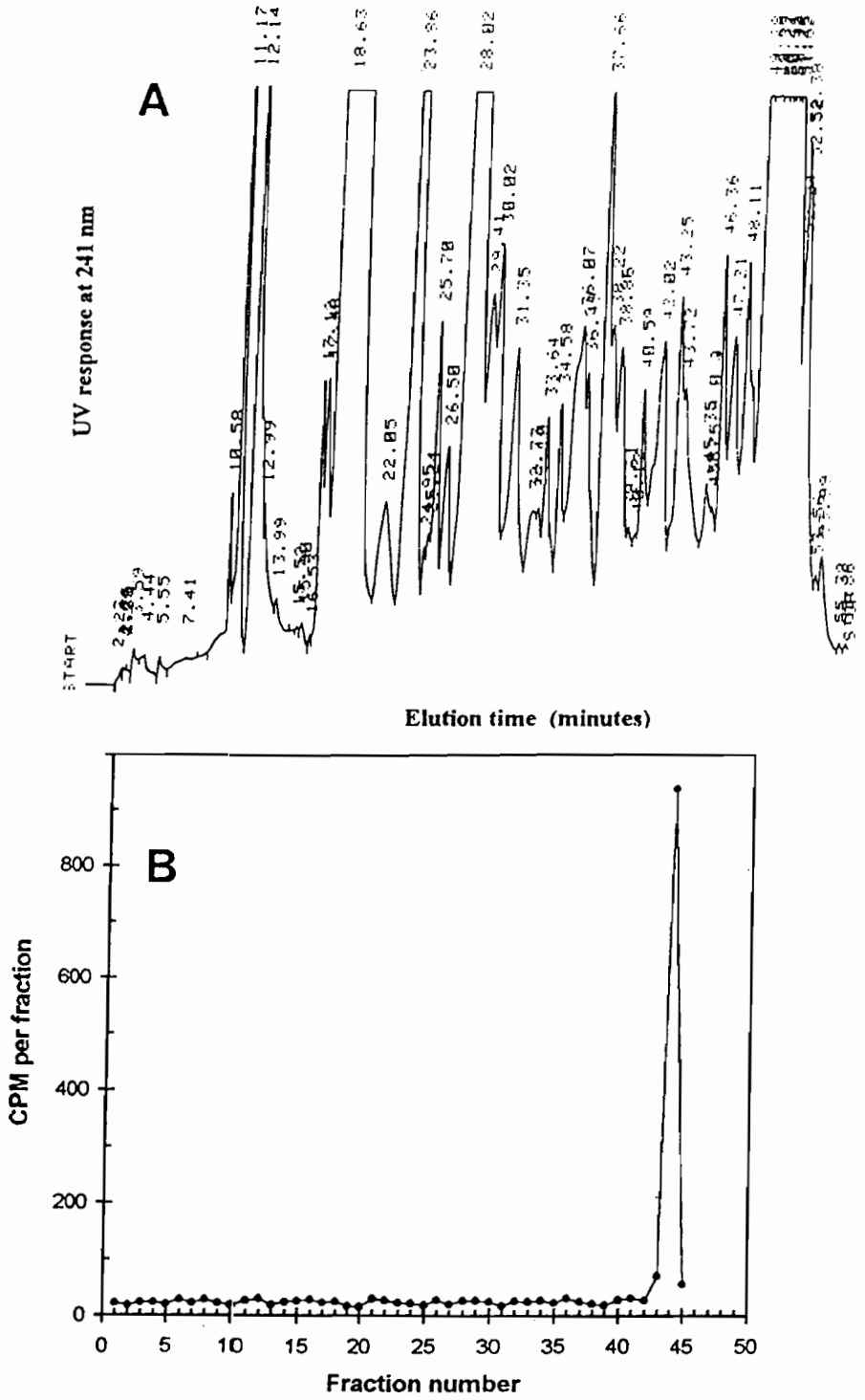

Fig. 4. Chromatographic analysis of combined fractions (fractions five to seven) collected from silica gel cleanup of a carcass sample; UV trace (A) and radiometric detection (B).

μl; peak width, 0.2 cm/min; and CPTH retention time, 5.8 to 7.5 min.

Bioconcentration factors (BCF) for the various fish tissues were determined by dividing the mean measured steady state [^{14}C] residue tissue values by the corresponding mean water concentration for the entire exposure period. The mean [^{14}C] residue tissue concentration was calculated using all individual tissue determinations, starting at the interval at which steady-state conditions were established through the end of the exposure period. A computer software program was used to provide theoretical BCF values for comparison with the actual experimental values that were obtained; this program solved uptake and depuration phase equations simultaneously for best-fit values for the depuration and uptake constants.

Metabolite identification

Tissue preparation. Duplicate treated and control samples of tissue (edible, viscera, and carcass), were weighed frozen and homogenized for 2 to 3 min in deionized water using a commercial blender; these samples were prepared from fish that had

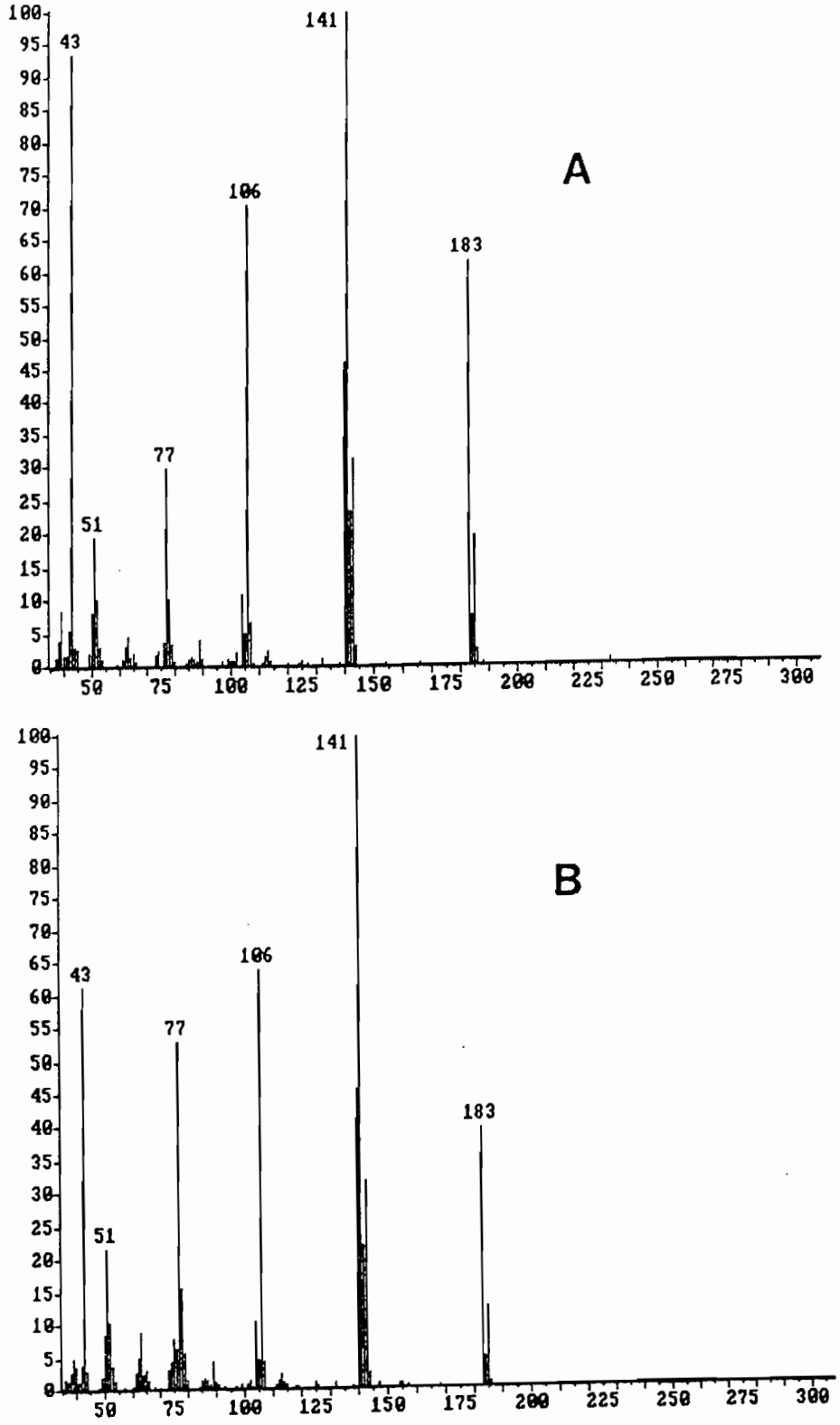

Fig. 5. Mass fragmentation spectra of the metabolite isolated from a carcass homogenate (A) and authentic *N*-acetyl-3-chloro-*p*-toluidine hydrochloride (B) prepared by acetylation of 3-chloro-*p*-toluidine hydrochloride.

been exposed to the radiolabeled chemical for 28 d. Sample weights ranged from 27 g (viscera) to 204 g (edible), with an overall yield of about 0.2 g tissue/g homogenate.

Fractionation of homogenates for radiochemical identification. Inasmuch as the radioactivity per milliliter of tissue homogenate was low, it was necessary to concentrate the samples by ethyl acetate extraction. Aliquots of the homogenates (100 ml) were extracted in 250-ml polypropylene bottles with 100 ml ethyl acetate and 75 g $(NH_4)_2SO_4$ by shaking each sample for 10 min using a mechanical shaker held in a walk-in cooler

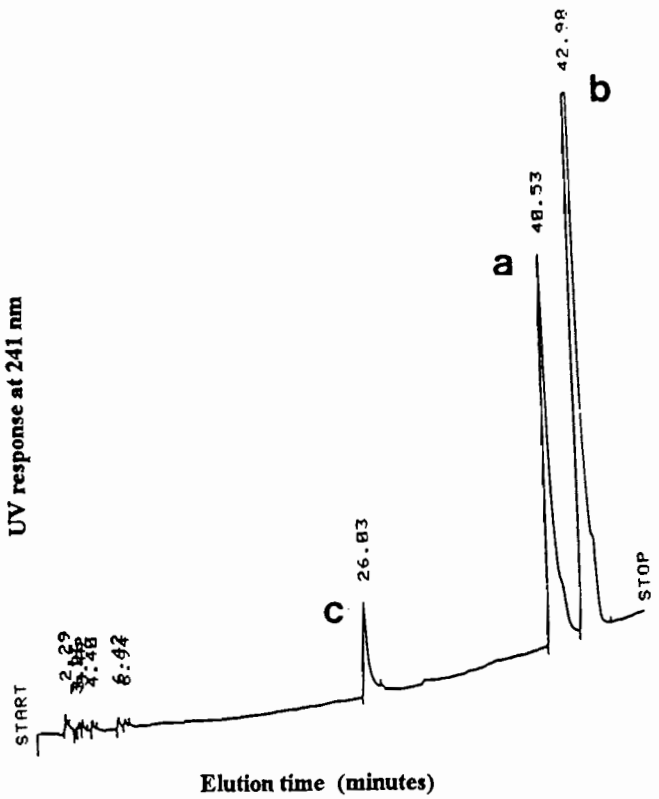

Fig. 6. Reverse-phase cochromatography of 3-chloro-*p*-toluidine hydrochloride (CPTH)(a), *N*-acetyl-CPTH (b), and impurity in *N*-acetyl-CPTH (c).

that was maintained at 4°C. Emulsions were eliminated by centrifuging the bottles for 30 min at 3,000 *g* in the cooler; this extraction was repeated two additional times. The ethyl acetate extracts were combined, dried using 15 to 20 g of anhydrous Na_2SO_4, and then reduced in volume in a water bath (40°C) under vacuum to about 1 ml. Because the resulting residue was oily and brownish orange in color, additional cleanup was required prior to attempting to isolate the [^{14}C]-labeled products by either TLC or HPLC.

The residue was dissolved in 10 ml of 5% ethyl acetate in hexane and passed through a small column (0.9 × 4 cm) containing 1 g of 40-μm silica gel, with a vacuum manifold used for solid-phase cartridge separations. Following sample application, the column was eluted with 10 ml of 5% ethyl acetate in hexane (four aliquots), followed by 20% ethyl acetate in hexane (four aliquots), and two final rinses of pure ethyl acetate. Each eluate was collected separately, evaporated to dryness in a water bath (40°C) under nitrogen, with the remaining residue dissolved in 2 ml of methanol : ethyl acetate (1:1). A 50 to 100 μl aliquot was then analyzed by LSC for total radioactivity. The remaining sample, in those fractions containing the majority of [^{14}C] activity, was evaporated to dryness and redissolved in methanol for TLC or in 50% methanol in water for reverse-phase HPLC.

For the TLC separations, 200 to 500 μl of the methanol solutions were applied as streaks (2–8 cm) to silica gel 60 plates containing fluorescent indicator (20 × 20 cm, E.M. Science) and developed for a distance of 10 cm using 9:1 chloroform : ethyl acetate. The plates were air-dried and autoradiograms developed by exposure to film (Eastman Kodak) for a period of 2 to 6 d.

With those samples to be separated by HPLC, the dissolved residues (300 μl of 60% methanol) were centrifuged at 16,000 *g* to clarify emulsions that formed; aliquots of these clarified solutions were injected into a Hewlett-Packard Model 1084 B HPLC with UV detection at 241 nm. Separation was accomplished on a C-18 column (250 × 4.6-mm i.d., Ultrasphere ODS, 5 μm, Beckman Instruments) at 35°C. The initial mobile phase contained 10% methanol in water, which was increased linearly to 80% methanol over 60 min at a flow rate of 1.0 ml/min. Column fractions were collected every minute in glass vials, with every three fractions pooled and counted for radioactivity.

Synthesis of N-*acetyl-CPTH.* An authentic sample of *N*-acetyl-3-chloro-*p*-toluidine was synthesized from the reaction of 3-chloro-4-methylaniline (Aldrich Chemical Co.) and acetyl chloride (Mallinckrodt) in diethyl ether, using standard procedures reported by Vogel [11]. The melting point of the resulting product (84–85°C) was consistent with that reported by Schofield (83°C) [12].

GC/MS confirmation. Sample residues for MS using an LKB mass spectrometer were derivatized by dissolving the sample in 30 μl bis-silyltrifluoroacetic acid (BSTFA) plus 1% trimethylsilane (TMS) and 5 μL pyridine. A 2-μl sample was gas chromatographed using a 30-m DB-5 column and temperature programing from 100 to 300°C at a temperature increase rate of 7°C/min.

RESULTS

CPTH bioconcentration

The [^{14}C] residues in the treated water solution as determined by LSC throughout the study period are presented in Table 1. As illustrated, the mean concentration of CPTH was about 0.1 μg/ml, which remained constant throughout the exposure period; this value compares favorably with that obtained using HPLC/UV (0.105 μg/ml). The radiopurity of delivered [^{14}C]CPTH on day 28 of the exposure was about 88 to 90% in the treated aquaria, as determined by HPLC/RAM analysis.

The extent of [^{14}C]CPTH absorption by the bluegill fish is presented in Table 1. As shown, the radioactivity increased in all tissues from day 1 to about day 7, at which point the [^{14}C] activity remained fairly constant. These data, subjected to statistical analysis, indicated no significant differences in the quantity of [^{14}C] residues in edible, nonedible, and whole-body tissues sampled at days 7, 10, and 14 of exposure, which strongly suggests that equilibrium or steady-state conditions were established by day 7. At day 7, the mean [^{14}C] residue tissue concentrations were 2.9, 12.0, and 7.5 μg/g for the edible, nonedible, and whole-body tissues, respectively. Analysis of control fish that were maintained throughout the 56-d study indicated that [^{14}C] activity was negligible, with no value exceeding an equivalent CPTH concentration of 0.17 μg/g.

Calculated BCF values were 33×, 150× and 88×, for edible, nonedible, and whole-body tissues, respectively, based upon a [^{14}C]CPTH continuous exposure concentration of about 0.1 μg/ml; these values compared favorably with predicted BCF values (computer program) of 40, 200, and 110 for the three respective types of tissues.

Metabolite identification

The distribution of radioactivity in edible tissue extracts, upon fractionation on silica gel columns, is illustrated in Fig. 1. Major amounts of [^{14}C] activity were present in eluates containing 20% ethyl acetate in hexane (fractions five to seven) and in fraction nine containing 100% ethyl acetate; similar distribution profiles

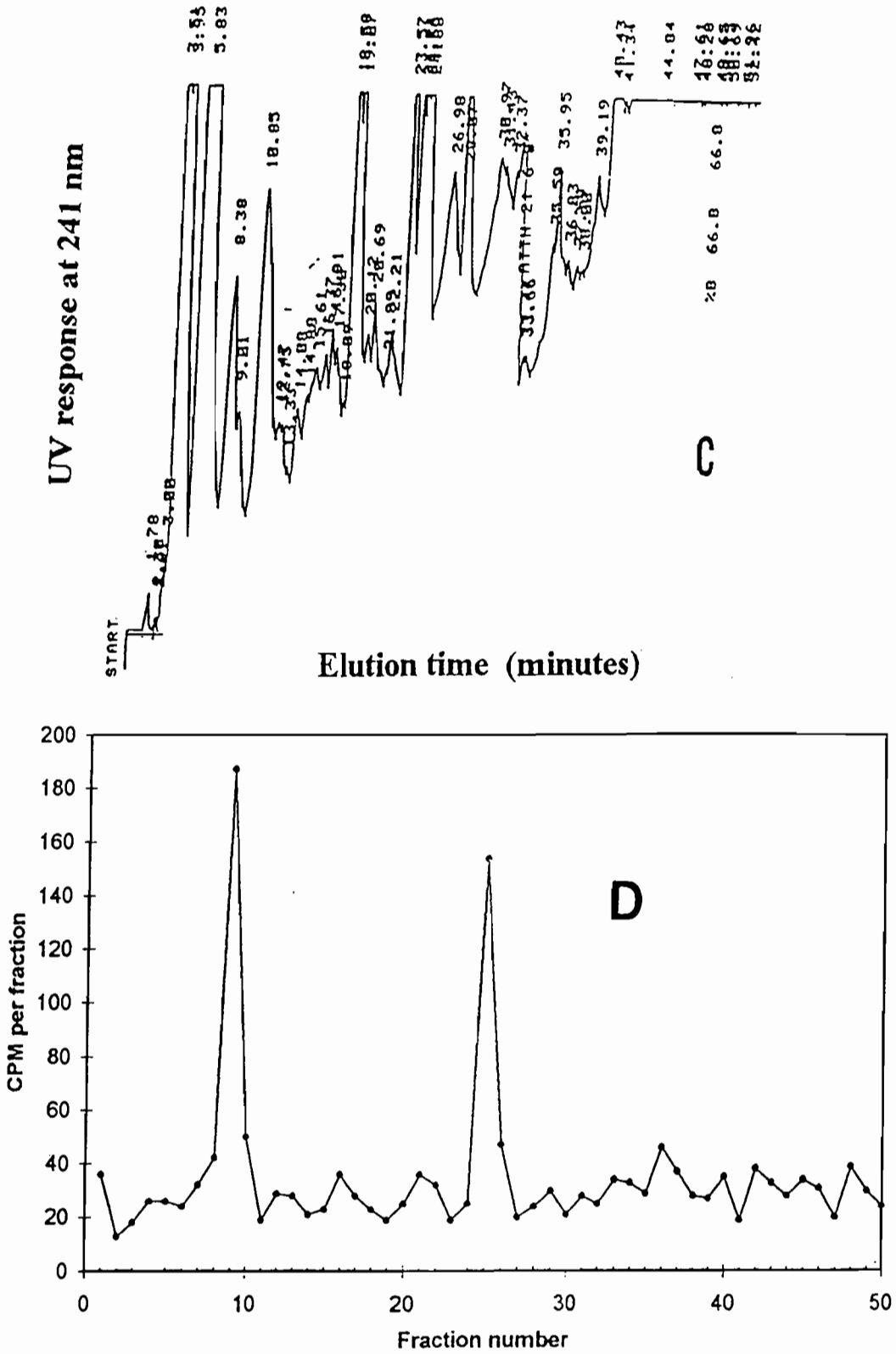

Fig. 7. The HPLC separation of silica gel cleanup fraction (fraction nine) from a carcass homogenate; UV detection (C) and radiometric detection (D).

were obtained upon fractionation of the viscera and carcass tissue extracts (Figs. 2 and 3). Recovery of radioactivity in fraction six, which was eluted with 20% ethyl acetate in hexane, ranged from 29% (edible) to 43% (carcass); with the viscera sample, 29% of the radioactivity eluted earlier in fraction five (Fig. 2). By eluting the columns with pure ethyl acetate, a second major fraction of radioactivity (nine) was recovered, which ranged from 15 to 38% for the three tissues. Total radioactivity that was recovered from the columns varied from 74 to 108% of the quantity applied. Recovery of radioactivity from control

tissue that was spiked with [^{14}C]CPTH and carried through the extraction and silica gel cleanup procedures demonstrated that the majority of [^{14}C] eluted in the first four fractions using 5% ethyl acetate in hexane.

Fractions six and nine of two tissue samples were analyzed by TLC followed by autoradiography. Fraction six from both the edible and carcass tissues showed a single radiochemical band that eluted behind CPTH with an R_F of about 0.2. Analysis of fraction five from a viscera extract resulted in a similar single radiochemical band. The radioactivity that eluted from the silica gel columns, using only ethyl acetate (fraction nine) remained essentially at the origin of the TLC plate; thus, this suggests that the compound(s) present in this fraction are more polar than that present in fractions five or six.

Results of HPLC analysis of the combined fractions (fractions five to seven) collected from the silica gel cleanup of a carcass sample are shown in Fig. 4. As illustrated in the UV trace (A), numerous absorbing peaks were present in this fraction, but only a single radiochemical peak (fraction 44) was detected (B). Under these gradient chromatographic conditions, CPTH eluted within 40 to 41 min, whereas the *N*-acetylated metabolite was retained slightly longer (43 min).

The radioactivity in fraction 44 was recovered by adding 7 ml of water to 3 ml of sample and passing the diluted sample through a methanol/water hydrated C-18 solid phase extraction cartridge (C 18 Bond Elute LRC®, Analytichem International, Harbor City, CA, USA). The cartridge was dried for 5 min using a vacuum manifold and the cartridge then rinsed with 3 ml of water followed by 2 ml of methanol. The resulting eluate was then evaporated to dryness and subjected to GC/MS. The resulting mass fragmentation pattern is shown in Fig. 5A together with the mass spectra of authentic *N*-acetyl-CPTH (Fig. 5B). The similarity of the spectra confirms that the metabolite was the *N*-acetyl breakdown product of CPTH. Further confirmation was obtained (Fig. 6) by HPLC (cochromatography) of authentic *N*-acetyl-CPTH with CPTH. As illustrated, the authentic acetyl metabolite eluted slightly later than CPTH, as did the radiochemical metabolite; thus, confirmation of identity was established.

The silica gel cleanup fraction nine (same carcass tissue homogenate) was also subjected to HPLC analysis using UV and radiometric detection. These results showed numerous absorbing peaks but only two radiochemical peaks, which was also suggested by the TLC data (Fig. 7). Moreover, there was no evidence of a radiochemical peak in the area that either CPTH or *N*-acetyl-CPTH elute. Attempts to obtain adequate quantities of these apparent metabolites sufficiently pure for GC/MS were unsuccessful. Derivatization of these products followed by GC/MS analysis showed that the fraction contained sugars and the mono-TMS derivative of phenylalanine (data not presented). However, products related to CPTH could not be identified.

DISCUSSION

Bluegill sunfish, exposed to 0.1 μg/ml of [^{14}C]CPTH for a 28-d exposure period, absorbed radioactivity equivalent to 3.5, 17.0, and 9.3 μg/g of the pesticide in edible, nonedible, and whole-body tissues, respectively. The mean steady-state tissue concentrations in these three tissues at day 7 were 2.9, 12.0, and 7.5 μg/g, respectively. These quantities of [^{14}C]CPTH, expressed as BCFs ranged from 33× to 150× in the edible and nonedible tissues; the whole-body tissue BCF was calculated as 88×. About 64% of the accumulated radiolabeled CPTH and metabolites were eliminated from the fish by day 28 of depuration. Essentially all of the [^{14}C] activity was present as the metabolites rather than the parent compound.

Three metabolites were found to be common to all fish tissues based upon chromatographic evidence. The major metabolite was identified as *N*-acetyl-CPT based upon mass spectrometry and chromatography, as compared to an authentic standard. Two minor polar metabolites were detected by TLC-autoradiography and HPLC with radiometric detection. Due to the presence of extensive coeluting materials with the radiometric peaks, mass spectral data could not be obtained for compound identification. However, based upon the chromatographic behavior, these metabolites may be 4-acetamido-2-chlorobenzoic acid and 4-amino-2-chlorobenzoic acid.

The *N*-acetylated derivative of CPTH has previously been reported by Peoples [13] to occur in mammals and birds, in addition to 4-acetamido-2-chlorobenzoic acid. Moreover, *N*-acetyl-CPTH has been reported to be less toxic to rats than CPTH, but twice as toxic to starlings on a molar basis [14,15]. In birds, *N*-acetyl-CPTH and 4-acetamido-2-chlorobenzoic acid were deacetylated by kidney homogenates, whereas rats exhibited very little deacetylase enzyme activity [16]. Based upon reported data and our study results, the metabolism of CPTH in fish may parallel that observed in birds and mammals.

A potential concern in the use of CPTH is the biomagnification of the compound in fish-eating birds (herons, kingfishers, etc.) and the resulting nephrotoxicity. Gulls have been shown to be effectively controlled by CPTH [5], but not by using fish as a source of bait. Although some given species (hawks) are devoid of the deacetylase enzyme necessary to induce nephrotoxicity [16], little information is available for other fish-eating birds.

Although the effects of biomagnification cannot be assessed at this time, our research demonstrates that when bluegill sunfish are continuously exposed to sublethal levels of CPTH, bioconcentration will not occur to an appreciable extent, and that residues will be rapidly excreted from the fish upon removal from the CPTH source. Furthermore, available ASTM information pertaining to various aquatic organisms indicates that bioaccumulation will not be a problem if the bioaccumulation factor is below 100 [17]. Also, other research that was conducted at our laboratory has shown that CPTH is rapidly dissipated in the environment by microbial degradation in soil (R.J. Spanggord et al., [18]), soil binding [19], and photochemical processes (D. Yao and T. Mill, unpublished data). Thus, any widespread ecological impact resulting from the proper use of CPTH appears to be small.

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